Urinary Markers of Nucleic Acid Oxidation and Long-Term Mortality of Newly Diagnosed Type 2 Diabetic Patients

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OBJECTIVE—We analyzed data from a cohort of 1,381 newly diagnosed type 2 diabetic patients to test the hypothesis that urinary markers of nucleic acid oxidation are independent predictors of mortality.

RESEARCH DESIGN AND METHODS—We examined the relationship between urinary excretion of markers of DNA oxidation (8-oxo-7,8-dihydro-2′-deoxyguanosine [8-oxodG]) and RNA oxidation (8-oxo-7,8-dihydroguanosine [8-oxoGuo]) and long-term mortality using Cox proportional hazards regression.

RESULTS—After multivariate adjustment, the hazard ratios for all-cause and diabetes-related mortality of patients with 8-oxoGuo levels in the highest quartile compared with those in the lowest quartile were 1.44 (1.12–1.85) and 1.54 (1.13–2.10), respectively. Conversely, no significant associations between 8-oxodG and mortality were found in the adjusted analyses.

CONCLUSIONS—Urinary excretion of the RNA oxidation marker 8-oxoGuo measured shortly after diagnosis of type 2 diabetes predicts long-term mortality independently of conventional risk factors. This finding suggests that 8-oxoGuo could serve as a new clinical biomarker in diabetes.

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Over the last decade, there has been an increased focus on the role of oxidative stress in the pathophysiology of diabetes-related complications. The diabetic state is associated with increased levels of markers of oxidative stress, and evidence derived from mechanistic studies suggests that oxidative stress has an important role in the pathogenesis of diabetes complications (1–7). Markers of intracellular oxidative stress that could be used as new biomarkers in diabetes are the oxidatively modified guanine nucleosides 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), which are widely used as markers of DNA and RNA oxidation, respectively. These markers can conveniently be assessed in the urine, which makes them well suited for use in risk stratification and therapy control.

The prognostic importance of urinary markers of oxidative stress in patients with type 2 diabetes is unknown. In the current study we used data from a population-based cohort of 1,381 patients newly diagnosed with diabetes to test the hypothesis that the urinary markers of oxidative stress 8-oxodG and 8-oxoGuo are independent predictors of mortality.

RESEARCH DESIGN AND METHODS

Study population

In the Diabetes Care in General Practice study (8), 474 general practitioners agreed to include all subjects on their practice list who fulfilled the following criteria: newly diagnosed diabetes based on hyperglycemic symptoms and/or raised blood glucose values, diagnosed between 1 March 1989 and 28 February 1992, and aged 40 years or over. Accordingly, a total of 1,543 newly diagnosed diabetic patients were eligible, but 162 were excluded because of protocol-based exclusion criteria: life-threatening somatic disease (50 patients), severe mental illness (50 patients), or unwillingness to participate (62 patients). This gave a final study population of 1,381 patients. Based on the onset of insulin treatment within 180 days of diagnosis, ~97.5% of the patients were considered to have type 2 diabetes (8).

The protocol was approved by the ethics committee of Copenhagen and Frederiksberg, and informed consent was obtained from all patients.

Baseline assessments

The participants produced a freshly voided morning urine sample as soon as possible after the day of diagnosis (median time from diagnosis to urine sample was 11 days; interquartile range, 4–29 days). Urinary markers of nucleic acid oxidation were measured at the Laboratory of Clinical Pharmacology (Rigshospitalet, Copenhagen). The urine samples, stored at −80°C until analysis, were assayed between 2009 and 2010 for the oxidatively modified guanine nucleosides 8-oxodG and 8-oxoGuo using a validated method of ultraperformance liquid chromatography and tandem mass

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spectrometry (9). 8-oxodG and 8-oxoGuo were normalized against urinary creatinine concentration.

Outcome assessments

The vital status of all the diabetic patients was certified on 1 January 2009 through the Danish Civil Registration System (10).

Diabetes-related death was defined as at least one of the following entries in the Danish National Register of Causes of Death (11): sudden death or death from myocardial infarction, stroke, renal disease, hyperglycemia, hypoglycemia, or peripheral vascular disease.

Statistical analysis

The associations between oxidative stress and all-cause mortality and diabetes-related mortality, respectively, were analyzed in Cox proportional hazards regression models based on time from diagnosis to death or censoring. Oxidative stress is represented by the natural logarithm of 8-oxodG and 8-oxoGuo, and by a four-class ordinal variable corresponding to the quartiles of the distribution. Three models were estimated for each of the oxidative stress variables and each of the outcomes: a univariate (unadjusted) model, a model adjusted for age and sex, and a multivariate model. Covariates included in the multivariate model were age, sex, smoking status, cohabitation status, physical activity, education, BMI, presence or absence of hypertension and of microalbuminuria, glycated hemoglobin, total cholesterol, triglycerides, serum creatinine, presence or absence of retinopathy and of peripheral neuropathy, and history of acute myocardial infarction and stroke.

RESULTS—Baseline characteristics according to quartiles of 8-oxodG and 8-oxoGuo are provided in Supplementary Tables 1 and 2. After a median follow-up period of 18.7 years (interquartile range, 18.2–19.2 years), 966 (70.0%) patients had died, of whom 584 were regarded as having had a diabetes-related death. The results of the unadjusted and adjusted Cox regression analyses are shown in Table 1. In the adjusted analyses, only 8-oxoGuo was significantly associated with mortality. The multivariate adjusted hazard ratios for all-cause and diabetes-related mortality of patients with 8-oxoGuo levels in the highest quartile compared with those in the lowest quartile were 1.44 (95% CI 1.12–1.85; P = 0.004) and 1.54 (1.13–2.10; P = 0.006), respectively.

When log 8-oxodG and log 8-oxoGuo were considered as continuous covariates, the results were similar to those described above. The multivariate adjusted hazard ratios for all-cause and diabetes-related mortality per unit increase in log 8-oxoGuo were 1.33 (95% CI 1.07–1.64; P = 0.009) and 1.40 (1.08–1.81; P = 0.01), respectively.

CONCLUSIONS—This study is, to our knowledge, the first to explore the association between urinary excretion of markers of oxidative stress and mortality in diabetic patients. Urinary excretion of the RNA oxidation marker 8-oxoGuo in a freshly voided morning urine sample shortly after diagnosis of diabetes independently

Table 1—*Relationship of 8-oxodG and 8-oxoGuo with all-cause and diabetes-related mortality*

Outcome	Unadjusted hazard ratio (95% CI)	*P*	Age- and sex-adjusted hazard ratio (95% CI)	*P*	Multivariate-adjusted hazard ratio (95% CI)*	*P*
All-cause mortality						
8-oxodG						
1st Quartile	1.0		1.0		1.0	
2nd Quartile	0.99 (0.83–1.19)	0.93	0.88 (0.73–1.07)	0.20	1.00 (0.78–1.28)	0.98
3rd Quartile	0.95 (0.79–1.14)	0.60	0.81 (0.67–1.97)	0.03	0.91 (0.71–1.15)	0.41
4th Quartile	1.35 (1.13–1.61)	0.001	1.00 (0.83–1.20)	0.99	1.07 (0.84–1.37)	0.58
Log 8-oxodG	1.30 (1.11–1.51)	0.001	1.12 (0.87–1.19)	0.82	1.01 (0.82–1.25)	0.91
8-oxoGuo						
1st Quartile	1.0		1.0		1.0	
2nd Quartile	1.01 (0.84–1.23)	0.88	0.89 (0.73–1.08)	0.23	0.96 (0.74–1.23)	0.73
3rd Quartile	1.37 (1.14–1.65)	<0.001	0.95 (0.79–1.15)	0.61	1.04 (0.81–1.33)	0.77
4th Quartile	2.05 (1.71–2.45)	<0.001	1.44 (1.19–1.74)	<0.001	1.44 (1.12–1.85)	0.004
Log 8-oxoGuo	1.97 (1.70–2.28)	<0.001	1.40 (1.19–1.65)	<0.001	1.33 (1.07–1.64)	0.009
Diabetes-related mortality						
8-oxodG						
1st Quartile	1.0		1.0		1.0	
2nd Quartile	0.99 (0.79–1.26)	0.97	0.88 (0.69–1.11)	0.28	1.08 (0.79–1.74)	0.63
3rd Quartile	0.95 (0.75–1.20)	0.68	0.78 (0.61–0.98)	0.04	0.99 (0.74–1.34)	0.97
4th Quartile	1.32 (1.05–1.66)	0.02	0.98 (0.77–1.23)	0.83	1.21 (0.89–1.65)	0.22
Log 8-oxodG	1.25 (1.03–1.53)	0.03	0.97 (0.79–1.18)	0.73	1.10 (0.85–1.42)	0.49
8-oxoGuo						
1st Quartile	1.0		1.0		1.0	
2nd Quartile	0.99 (0.77–1.28)	0.96	0.89 (0.69–1.14)	0.36	1.03 (0.74–1.39)	0.94
3rd Quartile	1.36 (1.07–1.73)	0.01	0.92 (0.72–1.17)	0.50	1.00 (0.73–1.36)	0.99
4th Quartile	2.13 (1.69–2.69)	<0.001	1.50 (1.18–1.91)	<0.001	1.54 (1.13–2.10)	0.006
Log 8-oxoGuo	2.03 (1.69–2.45)	<0.001	1.48 (1.21–1.82)	<0.001	1.40 (1.08–1.81)	0.01

*Covariates included in the multivariate model were age, sex, smoking status, cohabitation status, physical activity, education, BMI, presence or absence of hypertension and of microalbuminuria (urinary albumin ≥15 mg/L), glycated hemoglobin, total cholesterol, triglycerides, serum creatinine, presence or absence of retinopathy and of peripheral neuropathy, and history of acute myocardial infarction and stroke.

predicted all-cause and diabetes-related mortality, whereas the DNA oxidation marker 8-oxodG did not. The risk of death among those in the highest quartile of 8-oxoGuo excretion was nearly 50% higher than among those in the lowest quartile.

The prognostic information of 8-oxoGuo was independent of other characteristics of patients with type 2 diabetes that have been linked to mortality, most importantly age, sex, glycated hemoglobin, lipids, urinary albumin excretion, blood pressure, smoking, and pre-existing cardiovascular disease.

This observation suggests that measurement of urinary 8-oxoGuo provides additional information about risk and might be useful for identifying patients who would benefit the most from intensified treatment or specific treatment strategies, including nonpharmacological interventions. The combined use of 8-oxoGuo and other known risk factors in a multivariate risk factor approach could be useful for improving risk stratification of diabetic patients. 8-oxoGuo is a prime candidate for further investigation to establish the exact mechanisms responsible for its association with mortality in diabetic patients, and to evaluate its potential role as a clinical biomarker in diabetes.

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